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, Giri, A., Hellwege, J. N., Keaton, J. M., Park, J., Qiu, C., Warren, H. R., Torstenson, E. S., Kovesdy, C. P., Sun, Y. V., Wilson, O. D., Robinson-Cohen, C., Roumie, C. L., Chung, C. P., Birdwell, K. A., Damrauer, S. M., DuVall, S. L., Klarin, D., Cho, K., ... Wilson, P. W. F. (2019). Trans-ethnic association study of blood pressure determinants in over 750,000 individuals. *Nature Genetics*, *51*(1), 51-62. https://doi.org/10.1038/s41588-018-0303-9

Peer reviewed version

Link to published version (if available):
10.1038/s41588-018-0303-9

Link to publication record in Explore Bristol Research
PDF-document

This is the accepted author manuscript (AAM). The final published version (version of record) is available online via Nature at DOI: 10.1038/s41588-018-0303-9. Please refer to any applicable terms of use of the publisher.

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Genome, phenome and transcriptome study of blood pressure determinants in over 750,000 individuals

Ayush Giri[1,2]*, Jacklyn N. Hellwege[2,3]*, Jacob M. Keaton[2,3]* Jihwan Park[4]*, Eric S. Torstenson[2,3], Csaba P. Kovesdy[5], Yan V. Sun[6], Otis D. Wilson[2,7], Cassianne Robinson-Cohen[7], Christianne L. Roumie[8,9], Cecilia P. Chung[10], Kelly A. Birdwell[7,11], Scott M. Damrauer[12], Scott L. DuVall[13], Derek Klarin[14,15], Kelly Cho[16,17], Helen R. Warren[18,19], Evangelos Evangelou[20,21], Claudia P. Cabrera[18,19], Louise V. Wain[22], Chengxiang Qiu[4], Rojesh Shrestha[4], Brian S. Mautz[3], Muralidharan Sargurupremraj[23], Stéphanie Debette[23,24], Anne U. Jackson[25], Jian'an Luan[26], Jing-Hua Zhao[26], Sara M. Willems[26], Sébastien Thériault[27,28], Nabi Shah[29,30], Christopher Oldmeadow[31], Peter Almgren[32], Ruifang Li-Gao[33], Niek Verweij[34], Thibaud S Boutin[35], Massimo Mangino[36], Ioanna Ntalla[18], Nuno Sepúlveda[37], Tom G. Richardson[38], Elena Feofanova[39], James P. Cook[40], Aldi Kraja[41,42], Savita Karthikeyan[32], Najim Lahrouchi[43,44,45], Chunyu Liu[46], Philippe Amouyel[47], Martin Farrall[48], Neil Poulter[49], USSG[50], ICBP[51], BP-ICE[52], Patricia B. Munroe[18], Markku Laakso[53], Eleftheria Zeggini[54], Peter Sever[49], Praveen Surendran[32], Robert A Scott[26], Claudia Langenberg[26], Nicholas J Wareham[26], David Conen[55], Colin Neil Alexander Palmer[29], John Attia[56], Daniel I. Chasman[57], Paul M Ridker[57], Olle Melander[58], Dennis Owen Mook-Kanamori[33], Pim van der Harst[34], Francesco Cucca[59], David Schlessinger[60], Caroline Hayward[35], Tim D Spector[36], Marjo-Riitta Jarvelin[61,62], Branwen J. Hennig[63], Nicholas J. Timpson[38], Michael E. Matheny[9,64], Edward E. Siew[9,65], Cecilia Lindgren[43,66,67], Karl-Heinz Herzig[68], George Dedoussis[69], Joshua C. Denny[70], Wei-Qi Wei[70], Joshua C. Smith[70], Joanna M. M. Howson[32], Christopher Newton-Cheh[71], Mark J. Caulfield[17,18], Paul Elliot[19,72], J. Michael Gaziano[15,16], John Concato[73,74], Peter W.F. Wilson[75], Philip S. Tsao[76], Digna R. Velez Edwards[1,2], Katalin Susztak[4,76†], on behalf of the Million Veteran Program Chris O'Donnell[77†], Adriana M. Hung[2,7†Δ], and Todd L. Edwards[2,3†Δ]

* Indicates that these authors contributed equally to this work and share the first author position

† Indicates that these authors contributed equally to this work and share the senior author position

Δ Corresponding authors

Correspondence should be addressed to:

Adriana M. Hung

1161 21st Ave. S., MCN S-3223
Nashville, TN 37232
Vanderbilt University Medical Center
adriana.hung@vanderbilt.edu

Todd L. Edwards

Office 615, Suite 600
2525 West End Ave.
Vanderbilt University Medical Center
Nashville, TN 37204
todd.l.edwards@vanderbilt.edu

1) Department of Obstetrics & Gynecology, Vanderbilt Genetics Institute, Vanderbilt University Medical Center, Nashville, TN, USA
2) Biomedical Laboratory Research and Development, Tennessee Valley Healthcare System (626)/Vanderbilt University, Nashville, TN, USA
3) Division of Epidemiology, Department of Medicine, Institute for Medicine and Public Health, Vanderbilt Genetics Institute, Vanderbilt University Medical Center, Tennessee Valley Healthcare System (626)/Vanderbilt University, Nashville, TN, USA
4) Department of Medicine, Renal Electrolyte and Hypertension Division, University of Pennsylvania, Philadelphia, PA 19104, USA
5) Nephrology Section, Memphis VA Medical Center, Memphis TN, USA
6) Department of Epidemiology, Emory University Rollins School of Public Health; Department of Biomedical Informatics, Emory University School of Medicine, Atlanta, GA, USA
7) Division of Nephrology and Hypertension, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA
8) Department of Medicine, Vanderbilt University Medical Center, Nashville, TN , USA
9) Geriatrics Research Education and Clinical Center, Tennessee Valley Health System, Veteran's Health Administration, Nashville, TN, USA
10) Divisions of Rheumatology and Clinical Pharmacology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA
11) Division of Nephrology, Department of Medicine, Nashville Veteran Affairs Hospital, Nashville, TN, USA
12) Department of Surgery, Corporal Michael Crescenz VA Medical Center & Department of Surgery, Perelman School of Medicine, University of Pennsylvania; Philadelphia PA, USA
13) VA Salt Lake City Health Care System, University of Utah School of Medicine, Salt Lake City, Utah, USA
14) VA Boston Health Care System, Boston, MA; Center for Genomic Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
15) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA; Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
16) Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC), VA Boston Healthcare System, Boston, MA, USA
17) Division of Aging, Department of Medicine, Brigham and Women's Hospital, Boston, MA, Department of Medicine, Harvard Medical School, Boston, MA, USA
18) William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK
19) National Institute for Health Research Barts Cardiovascular Biomedical Research Centre, Queen Mary University of London, London, UK.
20) Department of Epidemiology and Biostatistics, Imperial College London, London, UK
21) Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Greece
22) Department of Health Sciences, University of Leicester, Leicester, UK
23) University of Bordeaux, Bordeaux Population Health Research Center, INSERM UMR 1219, F-33000 Bordeaux, France
24) Department of Neurology, Bordeaux University Hospital; F-33000 Bordeaux, France
25) Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI, USA
26) MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Cambridge, CB2 0QQ, UK
27) Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Canada
28) Department of Molecular Biology, Medical Biochemistry and Pathology, Laval University, Quebec City, Canada
29) Division of Molecular and Clinical Medicine, School of Medicine, University of Dundee, DD1 9SY, UK
30) Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad, 22060, Pakistan
31) Hunter Medical Research Institute, Newcastle, NSW
32) Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK

33) Leiden University Medical Center, the Netherlands
34) University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9700RB Groningen, The Netherlands
35) Medical Research Council Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, UK
36) Department of Twin Research and Genetic Epidemiology, Kings College London, London SE1 7EH, UK
37) Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, Keppel St, London WC1E7HT UK
38) MRC Integrative Epidemiology Unit (IEU), Bristol Medical School (Population Health Sciences), University of Bristol, Oakfield House, Oakfield Grove, Bristol, BS8 2BN, United Kingdom
39) Human Genetics Center, The University of Texas Health Science Center at Houston, Houston, Texas, USA
40) Department of Biostatistics, University of Liverpool, Liverpool, UK
41) Department of Genetics and Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St. Louis, MO, USA
42) Division of Statistical Genomics, Washington University School of Medicine, St. Louis, MO, USA
43) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA, USA
44) Cardiovascular Research Center Massachusetts General Hospital, Boston, Massachusetts; Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA, USA
45) Department of Clinical and Experimental Cardiology, Heart Center, Academic Medical Center, Amsterdam, The Netherlands
46) Boston University School of Public Health, Boston, USA
47) Institute Pasteur de Lille, Lille, France
48) Department of Cardiovascular Medicine, The Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, OX3 7BN, UK
49) International Centre for Circulatory Health, Imperial College London, W2 1PG, UK
50) Understanding Society Scientific Group, Supplemental Section for Full List of Authors
51) International Consortium for Blood Pressure
52) Blood Pressure-International Consortium of Exome chip studies
53) University of Eastern Finland, School of Medicine, Kuopio, Finland
54) Wellcome Trust Sanger Institute, Hinxton, CB10 1SA, UK
55) Population Health Research Institute, McMaster University, Hamilton, Canada
56) Hunter Medical Research Institute and Faculty of Health, University of Newcastle, Newcastle, NSW
57) Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA
58) Department of Clinical Sciences, Malmö, Lund University, Malmö, Sweden
59) Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, Monserrato, Cagliari, Italy
60) Laboratory of Genetics and Genomics, National Institute on Aging, NIH, Baltimore, MD, USA
61) Department of Epidemiology and Biostatistics, MRC–PHE Centre for Environment & Health, School of Public Health, Imperial College London, Norfolk Place, W2 1PG London, UK
62) Center for Life Course Health Research, Faculty of Medicine, University of Oulu, 90014 Oulu, Finland
63) Wellcome Trust, Population Health, London, UK
64) Departments of Biomedical Informatics, Medicine and Biostatistics, Vanderbilt University Medical Center, Nashville, TN, USA
65) Division of Nephrology and Hypertension, Vanderbilt Center for Kidney Disease, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA
66) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK
67) Big Data Institute at the Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, UK.
68) Institute of Biomedicine, Biocenter of Oulu, Medical Research Center; Oulu University and Oulu University Hospital, Oulu, Finland

69) Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University, Athens 17671, Greece
70) Department of Biomedical Informatics, Vanderbilt University Medical Center, Nashville, TN, USA
71) Cardiovascular Research Center and Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA, USA
72) MRC-PHE Centre for Environment and Health, Imperial College London, London, UK
73) Clinical Epidemiology Research Center (CERC), VA Cooperative Studies Program, VA Connecticut Healthcare System, 950 Campbell Avenue, 151B, West Haven, CT 06516, USA
74) Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA
75) Atlanta VAMC and Emory Clinical Cardiovascular Research Institute, Atlanta, GA, USA
76) VA Palo Alto Health Care System; Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA, USA
77) Department of Genetics, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, USA
78) VA Boston Healthcare, Section of Cardiology and Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

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ABSTRACT (100 Word Limit)

We conducted a genome-wide association study for blood pressure (BP) in 776,078 participants from the Million Veteran Program (MVP) and collaborating studies and discovered 207 novel loci from common-variant analyses and 54 rare variants. In a follow-up transcriptome-wide association study, we identified 3,036 associations between BP traits and genetically-predicted gene expression of 834 genes across 45 tissues. We identified pleiotropic effects from a phenome-wide association study and potential therapeutic targets from gene-drug relationships and toxicities. We also report structures from proximal tubule to collecting ducts that overexpress several BP gene homologs, using data from single-cell RNA sequencing in mice.

Decades of scientific evidence implicate elevated blood pressure (BP) in the etiology of cardiovascular disease, including coronary artery disease, peripheral arterial disease, and stroke, as well as renal, and ocular damage. Elevated BP accounts for at least 13% of annual deaths worldwide[1,2]. The risk of death from ischemic heart disease and stroke increases linearly for systolic blood pressure (SBP) greater than 115 mmHg and diastolic blood pressure (DBP) greater than 75 mmHg[3]. Recent treatment guidelines emphasize the benefit of BP-lowering strategies, including drug treatments, at lower thresholds of SBP or DBP[4]. These guidelines also identify a substantial patient population who are untreated or undertreated for elevated BP, or do not have the appropriate treatment response to anti-hypertensive drugs, highlighting the need to identify new gene targets for therapies[5].

Large-scale genome-wide association studies (GWAS) have collectively reported over 250 loci associated with BP traits[4-19], establishing that BP traits are complex with many genetic determinants of modest effects[6–24]. Large population studies of BP with collaborating cohorts and embedded meta-analyses provide an opportunity to identify genetic loci that are determinants of SBP, DBP and pulse pressure (PP) levels. In this trans-ethnic study, we meta-analyzed data for 318,891 Million Veteran Program (MVP) participants and 140,886 participants from the UK Biobank (UKB)[12]. We subsequently performed independent replication in 316,301 participants from the International Consortium for Blood Pressure (ICBP) and Vanderbilt University's BioVU cohort to study common variant associations with minor allele frequency (MAF) greater than 1% (Figure 1). We conducted two studies of rare variants, one focused on variants across the genome with independent replication in 458,577 participants from UKB and the other focused on exomic regions with replication in up to 417,143 participants from the Blood Pressure-International Consortium of Exome chip studies (BP-ICE) consortium. We extend findings in the literature by evaluating genetically predicted gene expression (GPGE) and related gene-drug relationships and toxicities, by conducting a phenome-wide association study (PheWAS) of BP genetic risk scores, and by performing functional studies to identify murine kidney cell types in which expression for implicated genes is enriched.

RESULTS

MVP participants (N = 318,891), representing the majority of the discovery sample size, were predominantly male (91.5%), and were administratively identified as whites (69.1%), with blacks and Hispanics representing 18.8% and 6.7% of the population respectively (Supplementary Table 1). Blacks were older on average [mean = 60.6 years, (standard deviation = 11.4)], followed by whites [58.9 (12.6)], and Hispanics [52.7 (14.5)]. Approximately half of the MVP participants were on antihypertensive medications and a quarter had diabetes. Participants from the UKB (N = 140,886) were also included in the discovery analysis; their characteristics are reported elsewhere[12].

Single Variant Analyses

<u>Common Variants</u>

We identified a total of 505 independent loci (207 novel loci, 298 previously reported) associated with one or more BP traits: SBP, DBP and PP. Among the previously reported loci 212 were

associated with SBP, 76 with DBP and 205 with PP (Table 1; Fig. 2a-c; Supplementary Tables 2a-c). Previously reported loci were not evaluated for further replication. Remaining loci were sought for replication with the ICBP consortium though meta-analysis to identify 207 novel loci. These included 128 loci for SBP, 4 loci for DBP and 126 loci for PP (Supplementary Tables 3a-c). Comparison of mean effect estimates of BP-trait increasing alleles showed that, on average, novel loci had smaller magnitudes of association than known loci (Table 1). The index SNPs at all independent loci from analysis of common variants explained 3.56%, 1.06% and 3.72% of the total variance for SBP, DBP and PP respectively. Novel variants contributed to 0.80%, 0.24% and 0.72% of the total variance explained by all independent loci for SBP, DBP, and PP, respectively.

Exonic Variants

Rare exonic variants with suggestive evidence of association (P-value = $5x10^{-6}$) from the discovery sample were queried for replication in populations from BioVU (N = 17,277) and the BP-ICE (N_{max} = 417,143) consortium. Eighteen variants were available for final meta-analysis. Ten missense variants from seven genes were associated with BP traits (Table 2). Five variants were associated with SBP and/or DBP (rs141328069 [*PDE3A*; Arg→Gln], rs139491786, [*SLC9A3R2*; Arg→Trp], rs61760904 [*RRAS*; Asp→Asn], rs73181210 [*PHC3*; Lys→Glu], rs3085380 [*DBH*; Gly → Ala]) with consistent directions of effect for SBP and DBP. Three rare variants from *COL21A1* (rs118079907 [Cys→Arg], rs200999181 [Gly→Val], and rs2764043 [Leu→Pro]) and one variant from *NOX4* (rs139341533; Leu→Phe) were significantly associated with PP but not with SBP or DBP and in fact had opposite directions effect for SBP and DBP. SNPs in *RRAS*, *DBH*, and one of the three SNPs in *COL21A1* (rs200999181) have been previously reported[22–24]. Average absolute values of effect estimates for SBP, DBP and PP in these variants were 1.52, 0.63, and 1.50 mmHg per allele, respectively.

Rare Variants

Discovery analysis in the MVP samples identified 1,684 rare variants with suggestive evidence for association across the three BP traits; 1,066 of these variants were available for meta-analysis from UKB. We observed statistically significant associations (P-value = $5x10^{-8}$) between 48 rare variants and one or more BP traits. We identified 40 SNPs for PP, eight SNPs for SBP, and two SNPs for DBP (Supplementary Table 4). Average absolute values of effect estimates for SBP, DBP and PP were 9.67, 2.33 and 13.89 mmHg per allele, respectively. The missense variants from *NOX4* (rs139341533), *SLC9A3R2* (rs139491786), and *COL21A1* (rs200999181, rs2764043) were evaluated in the both the exonic and rare-variant analyses separately (Table 2; Supplementary Table 4).

Transcriptome-Wide Association Analyses

Genetically-Predicted Gene Expression (GPGE) in Human Tissues

Common variants from the final meta-analysis were used to evaluate the associations between BP traits and GPGE levels across 44 Gene Tissue Expression Project (GTEx)[25] tissues using S-PrediXcan[26]. We identified statistically significant GPGE associations for 1,518 gene-tissue pairs

with SBP, 507 with DBP, and 1,938 for PP (Supplementary Tables 5a-c; Supplementary Figures 1a-c). We further identified 390 genes with this analysis that would not be identified if SNPs were annotated using the nearest gene. The proportion of significant associations that occurred in five cardiovascular tissues (aorta, coronary artery, tibial artery, left ventricle, and atrial appendage) were 16.7% for PP (P-value = 1.5×10^{-6}), 15.9% for SBP (P-value = 3.0×10^{-4}), and 15.6% (P-value = 5.4×10^{-2}) for DBP, an enrichment over the expected 11.4% if all tissues were represented equally. *MTHFR* was the top result from SBP and showed decreasing SBP with increasing GPGE in skeletal muscle, aorta, and several other tissues.

Colocalization of eQTLs and BP SNPs in Human Kidney

The GTEx reference data do not include sufficient numbers of kidney specimens to derive predictive weights for S-PrediXcan analysis. We applied the R package COLOC[27] to the kidney expression quantitative trait locus (eQTL) reference described by Ko et al[28] and our BP GWAS summary statistics to identify gene-SNP pairs with evidence for causal association for both gene expression and BP traits. We considered SNPs that reached genome-wide significance in the meta-analysis of common SNPs for each BP trait, and report 143 eQTL SNPs in 39 genes that had posterior probabilities of 80% or greater in either SBP, DBP or PP (Supplementary Tables 5a-c and 7). There was a high degree of consistency between results from COLOC and S-PrediXcan as 26 of 39 unique genes identified in the kidney were also observed across other tissues.

Murine Kidney Single Cell Sequencing Analysis

Homologs of human genes with greater than 80% posterior probability in colocalization analyses were further investigated for kidney cell type-specific RNA expression using single cell sequencing in murine kidney cells. Cells were clustered into 11 groups representing structural features and other cell types found in the kidney. Eighteen of the 25 genes that showed enrichment of expression in any of the murine kidney cell clusters were enriched in five tubule-related cell types: proximal tubules, loop of Henle, distal convoluted tubules, collecting duct peripheral cells and collecting duct intercalated cells (Figure 3; Supplementary Table 8a-c). Cross-referencing protein expression levels in the Human Protein Atlas[29] confirmed findings from murine kidney, including higher expression of *PNKD, SRR, SFXN2* and *CLCN6* proteins in tubules compared to glomeruli (Supplementary Table 9).

Assessment of Gene-Drug Relationships

To better understand how genes identified in the study relate to medications that directly or indirectly affect BP, associations identified from the GPGE analyses were investigated for enrichment of gene targets for known antihypertensive medications, non-antihypertensive medications, and medications with adverse drug events (ADEs) for hypertension and hypotension (Supplementary Tables 10-12). We did not observe a significant difference between the proportion of genes targeted by any drug (22.6% and 20.5% annotated by nearest gene or GPGE association, respectively; χ^2 P-value = 0.37), but found a significant difference in the proportion of genes targeted by antihypertensive drugs (5.2% and 21.7% for nearest gene or GPGE association, respectively; χ^2 P-value = 6.7×10^{-16}) (Supplementary Materials). We identify

175 gene-drug relationships between genes that increase BP with increasing GPGE and drugs that either inhibit or antagonize those genes (Supplementary Materials). The genes *PDE3A*, *PSMB9*, and *SH2B3* targeted by the non-antihypertensive drugs theophylline, carfilzomib, and pazopanib, respectively, have adverse drug events of either hypo- or hypertension and increase BP with increasing GPGE (Supplementary Materials). These genes are potential targets for anti-hypertensive drug development.

Phenome-Wide Association Study (PheWAS) with BP Genetic Risk Scores

To systematically evaluate pleiotropy between genetic predictors of BP-traits and diseases throughout the phenome, we performed PheWAS using BP-trait weighted genetic risk scores (GRS) in self-reported/administratively identified white individuals in the MVP. We used all known and novel common index SNPs from the final meta-analysis to generate weighted genetic risk scores (w-GRS) for each BP trait and regressed PheWAS outcomes from MVP onto those scores, adjusted for the top 10 principal components. Eighty eight of 1,813 phenotypes were significantly associated with any of the three GRSs at a Bonferroni correct threshold of $P < 2.76 \times 10^{-5}$ (Supplementary Table 13). Hypertension (smallest $P < 1 \times 10^{-305}$), essential hypertension (smallest $P < 1 \times 10^{-305}$) and hypertensive heart and/or renal disease (smallest $P = 3.3 \times 10^{-173}$) were the top three associations for each of the three w-GRS. Associations with phenotypes in the circulatory system (N = 52) accounted for more than 50% of the significant results. The phenotype groups with the next most associations were endocrine/metabolic (N = 28), genitourinary (N = 10) and hematopoietic (N = 6).

Among significant associations, 45 were significant for all three w-GRS, 10 were significant for both SBP and DBP, and 15 were significant for both SBP and PP, demonstrating substantial overlap between signals captured by genetically predicted BP traits (Supplementary Figure 2; Supplementary Table 13). Thirteen associations were significant only for the PP w-GRS, of which five were the diabetes sequelae ophthalmic manifestations, neurological manifestations, diabetic retinopathy, other abnormal glucose, and polyneuropathy. Four of the six hematopoietic associations were specific to anemias and were associated only with the PP w-GRS. Aortic and other aneurysms were only associated with the DBP w-GRS, but not with other w-GRS.

Convergence of Evidence

Hypothesizing that convergence of evidence from multiple types of investigation (common and rare single variants, predicted gene expression, single-cell sequencing expression enrichment, and drug query) may inform functionally relevant gene targets, we scrutinized genes for which evidence of association was seen in three or more investigations (Table 3) and highlight a few novel genes. Paroxysmal nonkinesigenic dyskinesia (*PNKD*) expression was enriched in cells derived from the murine loop of Henle and distal collecting tubules. Signals for this novel locus were consistently associated with SBP across all analyses: GWAS, GPGE (in the adrenal gland) and colocalization (human kidney) (Table 3, Supplementary Tables 2a, 5a, and 6a). RNA expression of leucine-rich repeat flightless-interacting protein 2 (*LRRFIP2*) was enriched in immune cells derived from murine kidneys, and was co-localized in human kidney.

Its predicted expression was inversely associated with PP in tissues derived from the aorta and tibial artery (Table 3, Supplementary Tables 5c, 6c, and 8c). We also detected SNPs associated with SBP in an intergenic region 131 kilobase (kb) downstream from the relaxin/insulin like family peptide receptor 2 gene (*RXFP2*; Table 3, Supplementary Table 2a). GPGE of *RXFP2* in the adrenal gland, was positively associated with SBP and PP (Supplementary Tables 5a and 5c). The drugs Relaxin and Seralaxin target *RXFP2* (Supplementary Table 11). We report associations between the novel intronic common SNP rs73080767 and PP, and a missense rare variant rs141325069 and SBP in the phosphodiesterase 3A (*PDE3A*) gene, as well as a positive association between GPGE and SBP in pancreas and subcutaneous adipose tissues (Table 3; Supplementary Tables 2c, 4c, and 5a). The *PDE3A* inhibitor theophylline used to treat chronic obstructive pulmonary disease has hypotension listed as a potential adverse drug event, which is consistent with the effects of increased gene expression in our analysis (Supplementary Tables 11 and 12). Novel intronic SNPs in the adenosine kinase (*ADK*) gene were associated with SBP and its GPGE were positively associated with SBP and PP in the aorta among other tissues (Supplementary Tables 5a and 5c).

By focusing on genes identified from multiple analysis types, including single-cell RNA sequencing in murine kidney cells, we also report six previously-reported gene loci from the literature that are partially or fully re-annotated to new genes (Table 3). For example, our analysis identifies, the *SRR* gene instead of the *SMG6* gene as a more plausible gene target for BP regulation. *SRR* gene expression was enriched in the proximal tubule of the mouse kidney, was identified by colocalization analyses in human kidney, and its expression was associated with SBP in a variety of tissues including atrial appendage, left ventricle, coronary artery, tibial artery, skeletal muscle, adrenal gland, and thyroid (Supplementary Tables 5a-c, 6a, 7, 8 and 9). Another previously-reported gene locus identified via the nearest gene annotation strategy of assigning relevant genes in the chromosome 10q24 region suggests Sideroflexin 2 (*SFXN2*) and SUFU negative regulator of hedgehog signaling (*SUFU*) as potential genes of interest[11] (Table 3). Although *SUFU* GPGE is associated with BP traits in various tissues, *SFXN2* GPGE was associated with BP traits in the left ventricle as well as human kidney, and its expression is enriched in mouse kidney tubule cell types (Supplementary Tables 5a, 5c, 6c, and 8c).

This study further characterizes the chromosome 1p36 region, which contains the methyltetrahydrofolate reductase (*MTHFR*) gene[30], along with several genes important for blood pressure regulation including natriuretic peptide A (*NPPA),* natriuretic peptide B (*NPPB)* and chloride voltage-gated channel 6 (*CLCN6*). In addition to common GWAS-significant SNPs, we observed a novel rare missense variant (rs202102042; Arg→Glu) in *NPPA* associated with PP (Table 2). *NPPB* GPGE was inversely associated with SBP and PP in the left ventricle, which is consistent with the known effects of this gene (Supplementary Tables 5a and 5c). *CLCN6*, a putative chloride antiporter[31], was expressed in the tubule cell types of the mouse kidney, the SNP rs12741980 was identified by colocalization analysis as potentially causal for both SBP and *CLCN6* expression in human kidney (Supplementary Table 6c). Among all known antihypertensive gene-targets, *CLCN6* was most significant, with an inverse association between its GPGE and SBP in the tibial artery tissue ($\beta = -2.76$, P-value = 8.14×10^{-45}; Supplementary Tables 5a and 10).

DISCUSSION

Here we present the results from multi-omic analyses of a multi-stage transethnic GWAS consortium for BP traits. By incorporating large sample sizes, bioinformatics methods, and external measures of gene expression from a model system, we have re-interpreted the genetic architecture of BP, and identified tissues and anatomical features where BP genes are relevant. We report distinct relationships between each genetically predicted BP trait and features of the clinical phenome. Interrogation of gene-drug relationships and toxicities for GPGE associations provides additional evidence for known and novel BP genes and suggests target genes for drug development and repurposing of existing drugs as BP treatments. We emphasize the utility of large-scale GWAS of BP traits as a requisite starting point for downstream analyses that provide insights into clinical factors, genetic etiology, pathophysiology, and pharmacology of BP homeostasis.

Convergent evidence from multiple analyses identified several novel BP genes including *RFPX2*, *PDE3A* and *ADK*. RXFP2 is a receptor for the pregnancy hormone relaxin[32], which causes vasodilation, increases cardiac output and renal perfusion, and has been evaluated in clinical trials as a treatment for acute heart failure, although the results of the latest trial did not demonstrate a benefit[33–35]. *RXFP2* is expressed in multiple tissues, which likely underlies the multiple physiological effects of the relaxin hormone throughout the circulatory system[36].

PDE3A is targeted by a wide variety of inhibitor drugs for which the indications vary and include congestive heart failure, hypertension, and heart disease. The *PDE3A* inhibitor theophylline used to treat chronic obstructive pulmonary disease has hypotension listed as a potential adverse drug event, which is consistent with the effects of increased gene expression in our analysis (Supplementary Tables 11 and 12). The autosomal dominant Mendelian condition Hypertension and Brachydactyly Syndrome (HTNB; OMIM: 112410) is caused by at least six distinct rare *PDE3A* mutations[37,38]. HTNB features include brachydactyly type E, severe salt-independent but age-dependent hypertension, increased fibroblast growth rate, neurovascular contact at the rostral-ventrolateral medulla, altered baroreflex blood pressure regulation, and death from stroke before age 50 years when untreated[39,40].

The product of *ADK*, adenosine kinase, catalyzes the transfer of gamma-phosphate from ATP to adenosine to form adenosine monophosphate and has widespread effects on multiple systems including cardiovascular, nervous and respiratory systems[41]. Adenosine has negative inotropic, chronotropic, and dromotropic effects on the heart; it terminates supraventricular tachycardia (SVT) involving the AV node and has been attributed with cardiac brady-arrhythmias[42,43]. Intravenous adenosine injection in humans induces vasodilation and systemic hypotension[44], and is the primary drug used in the treatment of stable narrow-complex SVT[45]. It is known to reduce blood pressure and blood pressure variability in rats and its actions are known to be mediated through adenosine receptors[46]. ADK reduces adenosine availability, and increased expression would be biologically predicted to increase BP. This prediction is in agreement with our findings where we show a positive association between GPGE of ADK and SBP and PP in aortic tissue (Supplementary Tables 5a and 5c).

A novel aspect of this study is the incorporation of single-cell RNA sequencing expression data from cells derived from murine kidneys. We show that BP genes identified by eQTL colocalization analyses in human kidneys are enriched in tubule cell types derived from murine kidneys. Expression was enriched for BP genes in tubule cell types, suggesting a significant portion of BP regulation in the kidney may be orchestrated in tubules by genes detected through GPGE associations.

Single-cell RNA sequencing in murine kidney identified genes for which expression was specifically enriched in immune cell types. For example, the protein encoded by *LRRFIP2* binds to the cytoplasmic tail of toll-like receptor 4 to activate the nuclear factor kappa b signal pathway. It is also known to downregulate the NLR Family Pyrin Domain Containing 3 (NLRP3) inflammosome by recruiting caspase-1 inhibitor flightless-1 to the inflammasome complex. Mice with spontaneous hypertension have increased levels of NLRP3 activation in the aorta and vascular smooth muscle cells[47]. Moreover, silencing of *NLRP3* ameliorates hypertension through reduced activation of the inflammosomes[47]. NLRP3 deficiency has been shown to improve gestational hypertension in pregnant mice without affecting intrauterine growth restriction[48]. High expression of NLRP3 inflammosomes have been shown in aortas of patients with atherosclerosis[49]. These evidence combined with our study findings strongly suggest *LRRFIP2* may reduce BP through downregulation of the NLRP3 pathway.

We examined genes enriched in murine kidney cell types and identified six previously-reported loci that were re-annotated by GPGE association (Table 3). Several lines of evidence implicate the *SRR* gene, while the nearest gene annotation strategy would identify the *SMG6* as associated with BP at this locus. A SNP in *SMG6*, rs216172, has been associated with coronary artery disease[50]; however, the SNP is an eQTL for *SRR* and not for *SMG6* (GTEx portal). *SRR* is an enzyme that catalyzes conversion of L-serine to D-serine, and eliminates water from L-serine to generate pyruvate and ammonia. The gene has also been associated with impaired cognitive function in mice[51–53], supporting a growing body of evidence that connects the association between hypertension and age-related cognitive decline[54,55].

This work helps to clarify the complex *MTFHR* gene locus by providing unique tissue-specific evidence for several genes in the region in relation to BP[30]. In addition to *MTFHR*, our study provides evidence for the role of *NPPA* (novel missense variant), *NPPB* (GPGE association with SBP and PP in the left ventricle), and *CLCN6* (SNP colocalization in human kidney, and enrichment in murine kidney). NPPA and NPPB are exclusively expressed in the heart and have biological functions that include natriuresis, diuresis, vasorelaxation, inhibition of renin and aldosterone secretion, and a key role in cardiovascular homeostasis[56]. The evidence for *CLCN6*, a putative chloride antiporter[31], was limited to analyses involving kidney tissues only. *CLCN6* is targeted by the antihypertensive medication chlorthalidone, and *NPPB* is targeted by the antihypertensive medication carvedilol, while *MTHFR* is not targeted by antihypertensive medications (Supplementary Materials). Findings for this locus highlight how effects of multiple associated genes from the same locus may vary by tissue type, and several nearby genes with very different biological functions may jointly contribute to the trait of interest.

Several other genes with strong biologic plausibility and empirical evidence worth highlighting include cysteine conjugate beta lysine 2 (*CCBL2*), FES proto-oncogene, tyrosine kinase (*FES*), and acetylcholinesterase (*ACHE*) (Table 3). GPGE of *CCBL2* in the tibial artery and adrenal gland was inversely associated with SBP (Supplementary Table 5a). The product of *CCBL2*, which encodes kynurenine aminotransferase 3 (KYAT3), is an aminotransferase that transaminates kynurenine to form kynurenic acid (KYA), a metabolite of tryptophan[57]. Direct administration of KYA in rats has been shown to reduce mean arterial pressure in spontaneously hypertensive rats, and microinjections of KYA block BP increases in glycine or glutamate injected rats[58,59]. Predicted expression of *FES* was inversely associated with SBP in the aorta, coronary artery and tibial artery tissues (Supplementary Table 5a). Naproxen, a non-steroidal anti-inflammatory drug, has an inhibitory effect on this gene (Supplementary Tables 11 and 12) and hypertension is one of its known side-effects[60]. ACHE terminates signal transduction at the neuromuscular junction by rapid hydrolysis of the acetylcholine released into the synaptic cleft[61]. Notably, inhibition of acetylcholinesterase is an effective treatment for orthostatic hypotension, especially in patients with supine hypertension[62]. Dimetacrine, a tricyclic antidepressant, and decamethonium, a muscle relaxant, have inhibitory effects on ACHE and are not currently prescribed as anti-hypertension medications.

To understand how genetically-predicted BP is associated with a diverse set of diagnoses, we calculated w-GRS weighted by estimates from each BP trait, and evaluated them with a PheWAS (Supplementary Table 13). The PP w-GRS was more likely to be associated with diabetic complications, including diabetic retinopathy and polyneuropathy, than w-GRS for SBP or DBP. PP is an independent predictor of cardiovascular disease and new-onset diabetes[63,64]. Elevated PP is a marker for arterial stiffness, which is positively associated with diabetic retinopathy and neuropathy[65]. Our finding of positive associations between the DBP w-GRS and aortic and other aneurysms support evidence from a previous study of 1.25 million individuals where a positive association between DBP and aortic aneurysms was reported[66]. Our study is the first to provide evidence for a genetic etiology for this reported association.

In conclusion, we applied multiple post-GWAS analyses and external resources to identify genes with effects on BP regulation. We report hundreds of novel SNPs and genes, previously-reported SNPs, and tissue-specific refinement of gene associations with meaningful directions of effects. We identified specific genes from gene-rich loci that are already targeted by antihypertensive drugs, and those already targeted by drugs with BP-related adverse events. Our study suggests that integrating tissue-specific expression into post-GWAS analysis provides greater insight into BP biology and potentially provides more actionable gene targets than the nearest gene approach.

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Acknowledgements

See accompanying document Acknowledgements_03282018_v2.docx

Disclosures/Conflicts of Interest

P.S received support from Pfizer Inc.

N.P received financial support from several pharmaceutical companies which manufacture either blood pressure lowering or lipid lowering agents, or both, and consultancy fees.

M.J.C. is Chief Scientist for Genomics England, a UK Government company.

The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the U. S. Department of Health and Human Services. This publication does not represent the views of the Department of Veterans Affairs or the United States Government.

Author contributions

Discovery analysis contributor: A.G., J.N.H., J.M.K., E.S.T., C.P.K., Y.V.S., O.D.W., C.R-C., C.L.R., C.P.Chung, K.A.B., H.R.W., E.E., J.M.M.H., M.J.C., P.E., M.E.M., E.E.S., J.M.G., J.C., P.W.F.W., P.S.T., D.R.V.E., C.O'D., A.M.H., T.L.E.

Replication study contributor: (ICBP) H.R.W., P.B.M., E.E., C.P.Cabrera, L.V.W., M.S., P.Amouyel, S.B., M.L., A.U.J., E.Z., M.J.C., P.E., P.Surendran, P.Sever, M.F., N.P., J.L., J-H.Z., S.M.W., R.A.S., C.Langenberg, N.J.W., D.C., S.T., C.N.A.P., N.Shah, C.O., J.R.A., D.I.C., P.M.R., O.M., P.Almgren, R.L-G., D.O.M-K, P.vdH., N.V., F.C., D.S., C.H., T.S.B, M.M., T.D.S, J.M.M.H; (BP-ICE) P.B.M., E.E., E.Z., P.Surendran, D.I.C., I.N., C.Lindgren, M-R.J., B.J.H., N.J.T., K-H.H., N.S., T.G.R., G.D., E.F., J.P.C., A.K., S.K., N.L., J.M.M.H., C.Liu, C.N-C.; (BioVU) J.N.H., D.R.V.E., T.L.E.

Central analysis: A.G., J.N.H., J.M.K., E.S.T., O.D.W., S.M.D., S.L.D., D.K., J.C.D., W-Q.W., J.C.S., D.R.V.E., A.M.H., T.L.E.

Human Kidney and Mouse model systems: J.P., C.Q., R.S., K.S.

Writing of the manuscript: A.G., J.N.H., J.M.K., C.P.K., Y.V.S., S.M.D., C.R-C., B.S.M., M.E.M., P.W.F.W., P.S.T., D.R.V.E., C.O'D., A.M.H., T.L.E.

Figure 1. Study design schematic. Flowchart depicting strategy for the three association analysis strategies (common, rare, and exonic variants), as well as replication selection criteria and numbers of samples and SNPs by stage. Subsequent TWAS and PheWAS analyses using common variant summary statistics are also presented.

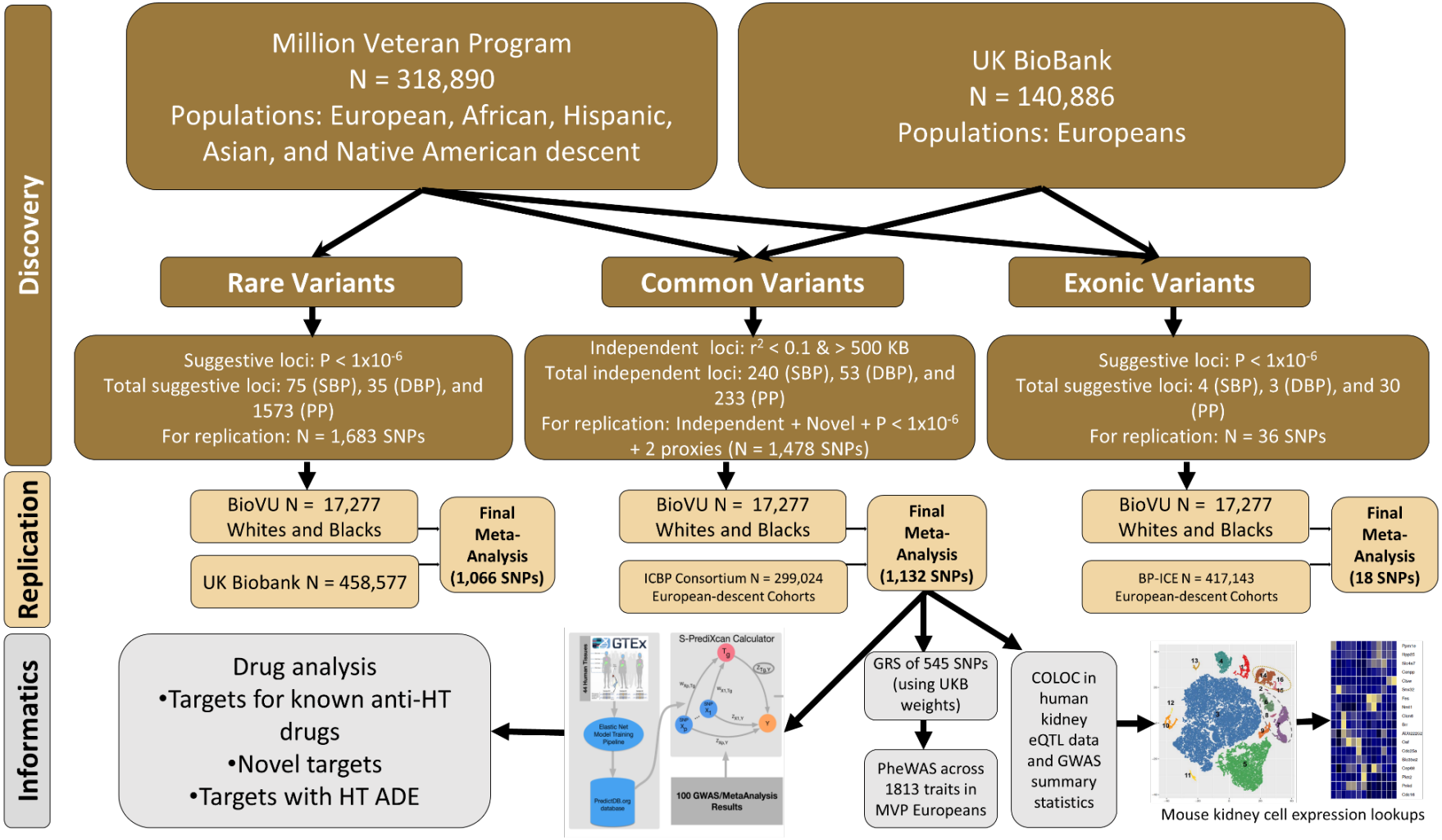

Figure 2. Manhattan plots summarizing GWAS results for (A) SBP, (B) DBP, and (C) PP.
Manhattan plot of the discovery+replication meta-analysis. The y axis shows the –log10 P-values and the x axis shows the chromosomal positions. The horizontal red line represents the thresholds of P-value = 5 $x10^{-8}$ for genome-wide significance. SNPs in red are in at previously identified loci (discovery only) whereas SNPs in orange are at novel loci which were significantly replicated

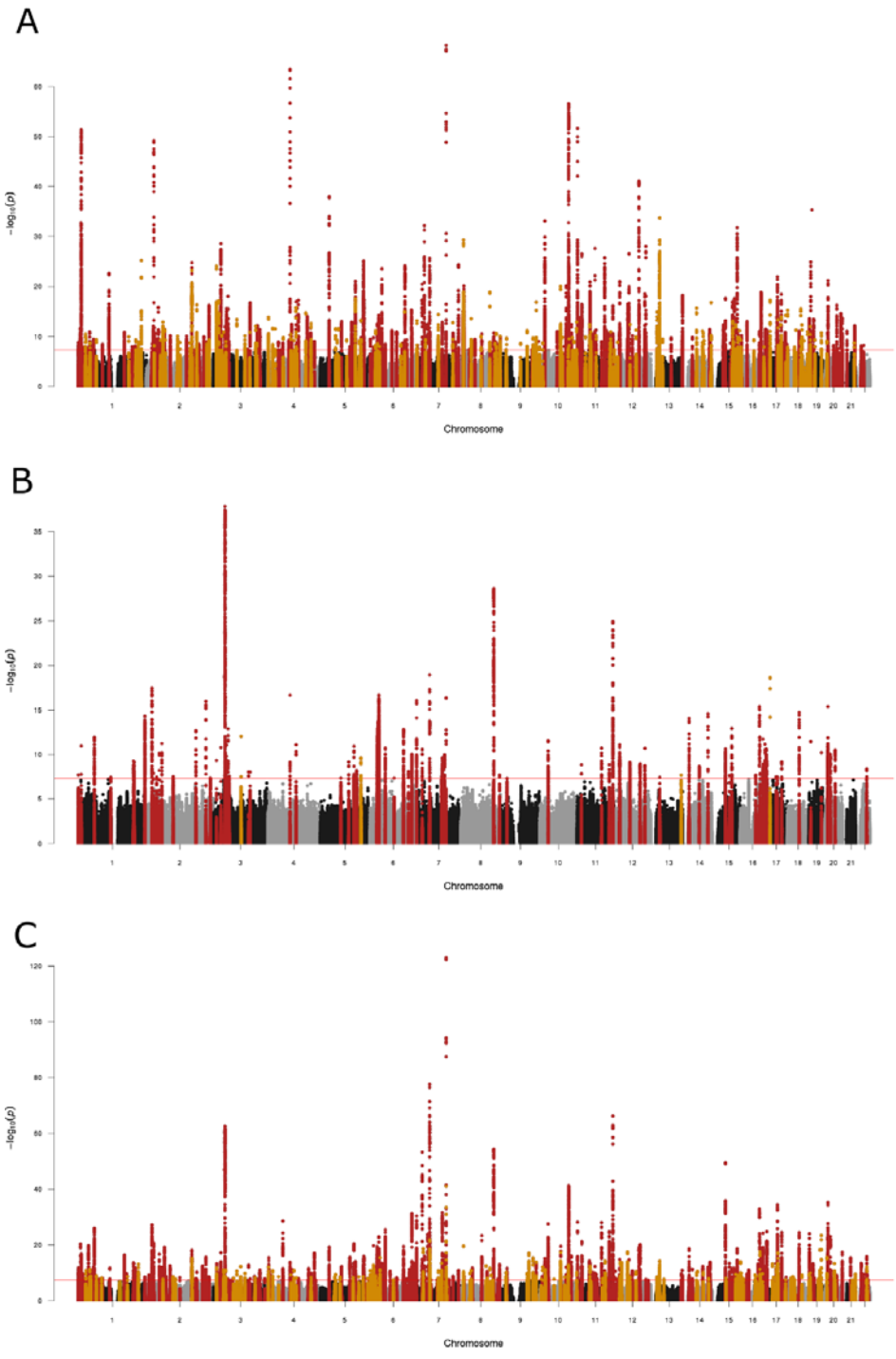

Figure 3. Mapping BP-associated genes to kidney cell type clusters. Average expression level of GWAS/eQTL defined genes. Mean expression values of the genes were calculated in each cluster. Color scheme is based on z-score distribution. Each row represents one gene and each column is single cell type cluster (as defined by Park et al.) on the heatmap. Endo: endothelial, vascular, descending loop of Henle, Podo: podocyte, PT: proximal tubule, LOH: ascending loop of Henle, DCT: distal convoluted tubule, CD-PC: collecting duct principal cell, CD-IC: CD intercalated cell, Fib: fibroblast, Macro: macrophage, Neutro: neutrophil, NK: natural killer cell.

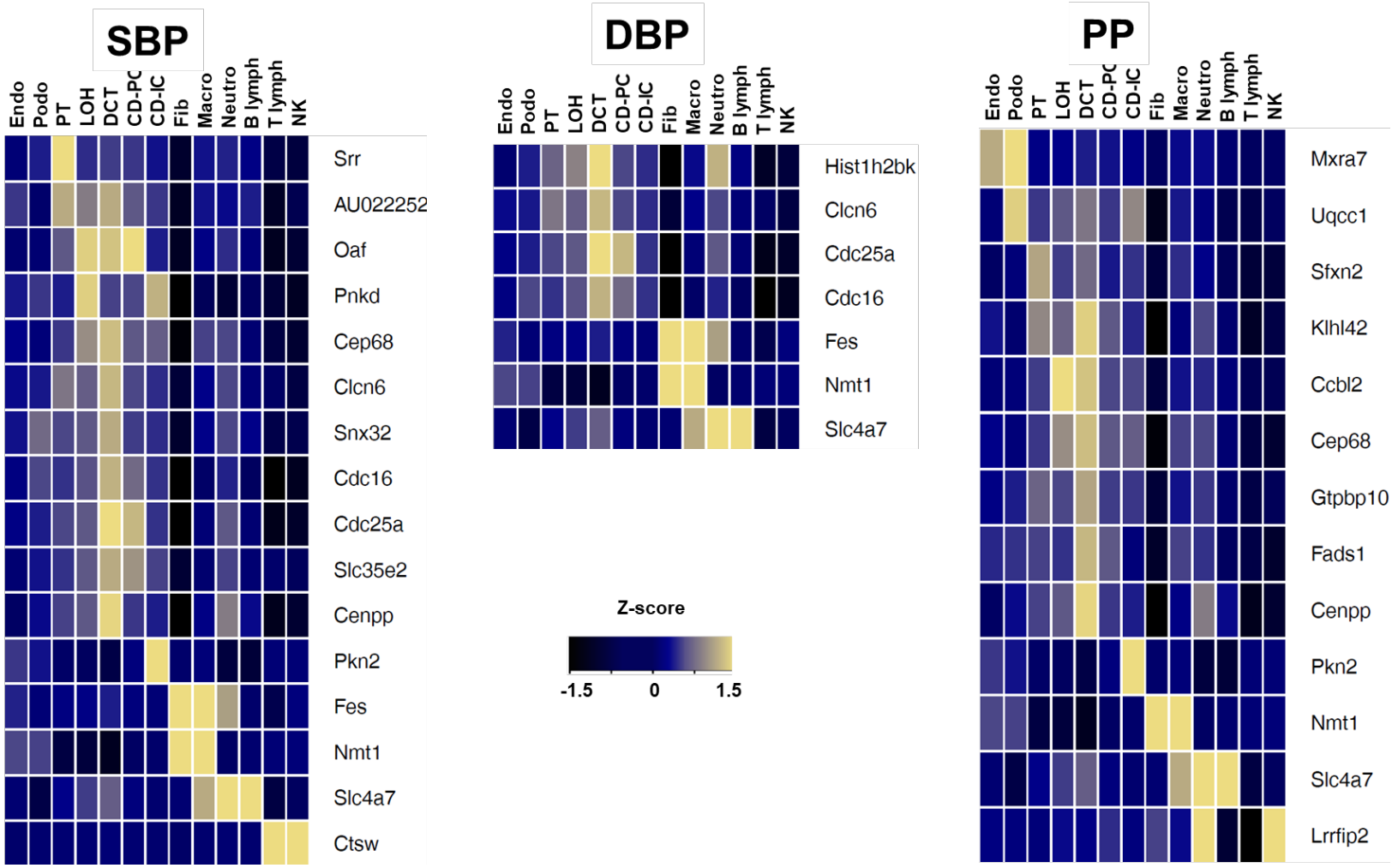

Table 1. Summary of known and novel loci achieving statistical significance from analysis of common variants.

	Known Loci		Novel Loci									
			All novel		P-value**	Tier 1		Tier 2		Tier 3		
	N Loci	Average Effect* (SD)	N Loci	Average Effect* (SD)		N Loci	Average Effect* (SD)	N Loci	Average Effect* (SD)	N Loci	Average Effect* (SD)
SBP	212	0.317 (0.151)	128	0.240 (0.117)	7.8x10-7	30	0.290 (0.154)	39	0.232 (0.124)	59	0.219 (0.075)
DBP	76	0.267 (0.146)	4	0.135 (0.016)	0.038	2	0.135 (0.022)	0	0	2	0.135 (0.002)
PP	205	0.269 (0.155)	126	0.176 (0.089)	8.3x10-10	21	0.189 (0.081)	47	0.180 (0.092)	58	0.168 (0.088)
Total	298	-	207	-		-	-	-	-	-	-

Known loci: known loci were only tested for significance in the discovery sample (N = 459,776). Novel loci: tested in discovery sample and ICBP (N-Discovery = 459,776; N-replication = 299,024; Total N = 758,800) *Mean beta = average and standard deviation of the absolute value of beta-estimates for each trait; **Represents P-value from two-sample t-test comparing mean beta for known loci and all novel loci. Tier 1 = First tier significance criteria: GWAS significance at discovery + replication passing Bonferroni threshold + consistent directions of associations between discovery and replication sets + GWAS significant at final meta-analysis; Tier 2 = Second tier significance criteria: GWAS significance at discovery + replication p-value < 0.05 + consistent directions of associations between discovery and replication sets + GWAS significant at final meta-analysis; Tier 3 = Third tier significance criteria: Suggestive significance at discovery + replication p-value < 0.05 + consistent directions of associations between discovery and replication sets + GWAS significant at final meta-analysis.

Table 2. Associations between missense variants identified in collaboration with consortia evaluating exonic variants and rare-variants.

SNP	Chr:BP	Gene	Amino Acid Change	EA/ RA	EAF	SBP			DBP			PP		
						Effect	P-value	N_{eff}	Effect	P-value	N_{eff}	Effect	P-value	N_{eff}
rs141325069	12:20769270	*PDE3A*	R-->Q	A/G	0.0030	1.415	**$8.7x10^{-9}$**	700771	0.716	$2.7 x10^{-5}$	700575	0.759	$5.2x10^{-5}$	700391
rs139491786#	16:2086421	*SLC9A3R2*	**R-->W**	**T/C**	0.0068	-1.917	**$4.6x10^{-21}$**	651069	-1.323	**$1.9x10^{-22}$**	651707	-0.643	$1.9x10^{-5}$	649540
rs61760904*	19:50139932	*RRAS*	**D->-N**	**T/C**	0.0073	1.160	**$1.1x10^{-12}$**	844155	0.523	$8.8x10^{-7}$	843327	0.668	**$2.0x10^{-8}$**	843773
rs73181210	3:169831268	*PHC3*	K-->E	T/C	0.0107	0.855	**$3.7x10^{-8}$**	845000	0.606	**$1.8x10^{-9}$**	844834	0.230	$4.2x10^{-2}$	842255
rs3025380*	9:136501756	*DBH*	G-->A	C/G	0.0046	-1.137	**$1.9x10^{-8}$**	864699	**-0.825**	**$7.2x10^{-10}$**	863755	-0.321	$3.0x10^{-2}$	864042
rs139341533#	11:89182666	*NOX4*	**L-->F**	**A/C**	0.0037	-0.814	$2.1x10^{-4}$	851884	0.223	$1.2x10^{-1}$	850481	-0.928	**$8.7x10^{-9}$**	852260
rs115079907	6:55924005	*COL21A1*	C-->R	T/C	0.0023	1.259	$2.1x10^{-3}$	837965	-0.582	$3.2x10^{-2}$	831917	1.703	**$1.4x10^{-8}$**	830745
rs200999181*#	6:55935568	*COL21A1*	**G-->V**	**A/C**	0.0014	1.900	$9.2x10^{-6}$	724111	-1.038	$4.3x10^{-4}$	718225	2.964	**$3.3x10^{-21}$**	724615
rs2764043*#	6:56035643	*COL21A1*	**L-->P**	**A/G**	0.0016	-1.532	$1.9x10^{-5}$	812384	0.353	$1.4x10^{-1}$	814667	-1.925	**$2.4x10^{-13}$**	810623
rs138582164	8:95264265	*GEM*	R-->.	A/G	0.0011	3.247	$2.1x10^{-7}$	633292	0.125	$7.6x10^{-1}$	632884	3.159	**$6.0x10^{-12}$**	632845
rs202102042^	1:11907171	*NPPA*	R-->Q	T/C	0.0004	3.863	$3.1x10^{-7}$	765853	1.058	$3.1x10^{-2}$	765853	3.198	**$2.4x10^{-8}$**	765853

*SNPs have been previously reported in the literature. #SNPs were identified in two different replication strategies: exonic set and rare-variant set.
^ SNPs were replicated in the rare variants replication set. For SNPs that were available in both rare-variant and exonic analyses, table reports results with the largest sample size.

Table 3. Converging evidence across analyses.

Gene	Novel	Index SNPs (Common; Rare)	Nearest Gene	CVD Tissues (S-PrediXcan)	Human Kidney (COLOC) Top eQTL	Mouse Kidney Single Cell Clusters	Related Drugs
OAF	Yes	rs896693	OAF	↑;-;-;-;-;-	rs508205	Tubules	-
PNKD	Yes	rs1870123	PNKD	-;-;-;-;-;↓	rs3731861	Tubules	-
SNX32	Yes	NA	NA	-;-;-;-;-;-	rs694994	Tubules	-
CTSW	Yes	NA	NA	-;-;-;-;↓;-	rs694994	Immune	-
CENPP	Yes	rs2761679	CENPP	-;-;-;-;-;-	rs10120915	Tubules	-
MXRA7	Yes	rs7219390	MXRA7	-;-;-;-;-;-	rs2286590	Glomerulus	-
UQCC1	Yes	rs6142381	UQCC1	↓;-;-;-↓;-	rs2425056 [b]	Glomerulus	-
KLHL42 [c]	Yes	rs571463591	RN7SKP15	-;-;-;-;↓;-	rs10842999 [b]	Tubules	-
LRRFIP2 [c]	Yes	rs7632108	GOLGA4	-;-;↓;-;↓;-	rs7632108	Immune	-
HIST1H2BK	Yes	NA	NA	-;-;-;-;-;-	rs1102565	Tubules	-
GTPBP10	Yes	rs10270950	STEAP	-;-;-;-;-;-	rs10953006	Tubules	-
SRR [c]	No	rs12952051	SMG6	↑;↑;-;↑;↑;↑	rs1048483	Tubules	pyridoxal phosphate
CEP68	No	rs111524356	CEP68	↑;↑;-;-;↑;-	rs1009358b	Tubules	-
CLCN6 [e]	No	rs6669371; rs202102042	CLCN6 NPPA	-;-;-;-;↓;-	rs12741980	Tubules	chlorthalidone; vincristine
CDC16	No	rs11617448	CDC16	↑;↑;↑;↑;↑;↑	rs61972052 [b]	Tubules	-
CDC25A [c]	No	rs35979968	MAP4	↓;↓;↓;↓;↓;-	rs71323396 [b]	Tubules	-
SLC35E2 [c]	No	rs1014988	NADK	-;-;-;-;-;-	rs7546498	Tubules	-
PKN2 [cd]	No	rs6679817	GTF2B	-;-;-;-;-;-	rs4142614	Tubules	-
CCBL2 [cd]	No	rs6679817	GTF2B	-;-;-;-;↓;↓	rs10922478 [b]	Tubules	-
FES	No	rs2071382	FES	-;-;↓;↓;↓;-	rs2521498 [b]	Immune	naproxen
NMT1	No	rs12951622	NMT1	-;-;↑;↑;↑;-	rs7405669 [b]	Immune	-
SLC4A7	No	rs2643826	SLC4A7	-;-;↓;-;-;-	rs9845896 [b]	Immune	-
SFXN2 [c]	No	rs17114641	SUFU	-;↑-;-;-;-	rs11191356	Tubules	-
FADS1 [c]	No	rs7125196	MIR4488	-;-;-;-;-;-	rs174530	Tubules	icosapent
RXFP2	Yes	rs9603376	*RXFP2*	-;-;-;-;-;↑	NA	NA	relaxin; serelaxin
NPPB [e]	No	rs202102042	*NPPA*	-;↓;-;-;-;-	NA	NA	carvedilol

NPR3	No	rs12656497	*NPR3*	-;-;-;-;-;↑	NA	NA	nesiritide
PDE3A	Yes	rs73080767; rs141325069	*VALUE MISSING*	-;-;-;-;-;-	NA	NA	theophylline
COL21A1 [a]	No	rs12203179; rs2764043	*COL21A1*	-;-;-;-;↑;-	rs61524954	NA	-
ACHE [ac]	No	rs138475089	*GNB2*	-;-;↑;↑;↑;↑	NA	NA	dimetacrine; decamethonium
ULK4 [a]	No	rs6797165	*ULK4*	-;↑;↑;↑;↑;↑	rs55835726	NA	-

Gene = Gene showed enriched expression in one or more cell type in murine kidney single-cell RNA sequencing experiment, or was significant in genetically predicted gene expression analysis for any of the 45 tissues; **Novel** = Indicator variable denotes whether variants in a given gene region have previously been reported in genome-wide association studies of BP traits. **Index SNPs** = Index SNP from common or rare variant analyses from each independent locus. **Nearest Gene** = Column reports genes that would have been identified if the nearest gene annotation strategy was used to link GWAS significant variants. **CVD Tissues** = Column identifies genes that were significantly associated with genetically predicted gene expression and BP traits (using S-PrediXcan) in the following cardiovascular related tissues: atrial appendage, left ventricle, aorta, coronary artery, tibial artery and adrenal gland. Up (down) arrow indicates a positive (negative) association between GPGE and at least one BP trait. Dash indicates data was not significant or unavailable. For a full-set of tissue specific results, see Supplementary Tables 5a-5c. **Mouse kidney single-cell clusters** = Column highlights the cell-type in which specific genes were enriched in murine single-cell RNA sequencing expression analyses. **Related Drugs** = Column summarizes drug targets which were identified to interrelate with the gene from xyz databases.

[a] Genetically predicted expression of these genes were positively associated with SBP and PP, but inversely associated with DBP; for all other genes directions of associations were consistent across traits.

[b] Gene had several significant eQTLs; presented SNP represents the most significant eQTL.

[c] Mismatch between post-GWAS expression based gene identification and nearest gene annotation strategy

[d,e] Genes are in the same locus

ONLINE METHODS

We conducted a multi-stage GWAS of common and rare variants in over 700,000 participants. We then performed additional bioinformatics analyses of GPGE for BP traits, evaluated cell types where associated genes are expressed, performed a phenome-wide association study of genetic risk scores for BP traits from the electronic health records of MVP participants, and screened known drugs to evaluate potential for repurposing and validate observed associations. A flow chart for analyses is presented in Figure 1.

Discovery Cohorts

The Million Veteran Program

The Million Veteran Program (MVP) is a large cohort of fully-consented participants who were recruited from the patient populations of 63 Department of Veterans Affairs (VA) medical facilities. Recruitment began in 2011 and is conducted in-person, which is initiated by an invitation letter and completed by answering baseline and lifestyle questionnaires, providing a blood sample, providing access to medical records, and giving permission for re-contact. Consent to participate is provided after counseling by research staff and mailing of informational materials. All documents and protocols have been approved by the VA Central Institutional Review Board. Blood samples are collected by phlebotomists and banked at the VA Central Biorepository in Boston, MA. Genotyping was conducted using a customized Affymetrix Axiom Biobank Array chip with additional content added to provide coverage of African and Hispanic haplotypes, as well as markers for common diseases in the VA population. Researchers are provided with de-identified versions of these data, and do not have the ability or authorization to link these details with a participants' identity.

MVP Genotype QC

Blood samples drawn from consenting MVP participants were shipped to the VA Central Biorepository in Boston, MA, where DNA was extracted and shipped to two external genotyping centers for genotyping on an Affymetrix Axiom Biobank array designed specifically for the MVP. The MVP genomics working group applied standard quality control and genotype calling algorithms to the data in batches using the Affymetrix Power Tools Suite (v1.18). Standard quality control pipelines were used to exclude duplicate samples, samples with more heterozygosity than expected, samples with an excess (>2.5%) of missing genotype calls, and samples with discordance of genetically inferred sex versus self-report. Related individuals (halfway between 2nd and 3rd degree relatives or closer) as measured by the KING software[67] were also excluded. Prior to imputation, variants that were poorly called or that deviated from their expected allele frequency based on reference data from the 1000 Genomes Project[68] were excluded. After pre-phasing using EAGLE v2[69], genotypes from the 1000 Genomes Project[68] phase 3, version 5 reference panel were imputed into Million Veteran Program (MVP) participants via Minimac3 software[70]. Principal component analysis was performed using the FlashPCA[71], to generate the top 10 genetic principal components explaining the greatest variability.

Race/Ethnicity

Information on race (whites, blacks, Asians, and Native Americans) and ethnicity (Hispanic: Yes or No) were obtained based on self-report through centralized VA data collection methods using

standardized survey forms, or through the use of information from corporate data warehouse (CDW), or Observational Medical outcomes Partnership (OMOP) data, when information from self-report survey was missing. Race and ethnicity categories were then merged to form the following administratively assigned race/ethnicity variables: non-Hispanic whites (whites), non-Hispanic blacks (blacks), non-Hispanic Asians (Asians), non-Hispanic Native Americans (Native Americans) and Hispanics. Individuals for whom race and ethnicity could not be confidently assigned due to conflicting records and missing data, were categorized as unknown. Prior to analysis QC, there were 15,710 Veterans with unknown status for race/ethnicity. For these individuals, we used a K-means clustering approach in R following the McQueen algorithm with the top 10 genetic principal components as input variables. In order to obtain the most reliable cluster designations for the missing data, the k-means approach was applied to the maximum available samples: the 1000 Genomes reference populations and all individuals for whom PCs were available regardless of whether race/ethnicity designations were unknown. K-clusters were optimized by testing values K=2 through K=10. K = 4 was ultimately chosen as the most optimal value, as visual examination of these most closely corresponded to whites (N=5,265), blacks (N=4,671), Asians (N= 3,936) and Hispanics (N= 1,838).

MVP BP Phenotypes

We selected adults (age ≥ 18) and used the earliest median eligible non-Emergency Department outpatient measured SBP in the EHR, and also used the corresponding DBP from this measure. Measures are ineligible if they occur at or after an ICD-9 code from the groups 585, 405, or 428. If pain scores were available, we censored BP measures taken during encounters when a pain score ≥ 5 was recorded, because severe pain can elevate BP[72,73]. For measures taken while a patient was on an antihypertensive medication we added 15 mmHg to SBP and 10 mmHg to DBP[8,74].

MVP Analysis

For the MVP GWAS we performed linear regression association tests with additive models for untransformed BP traits, after adjusting for medication use. We adjusted linear regression models analyzing SNP associations for age at BP measure, age^2, sex, BMI measured within 1 year of BP measure, and the top 10 genetic principal components in analyses. All primary analyses for the MVP were conducted by either strata of administratively assigned race/ethnicity or by their empirically designated clusters. All regression based analyses were conducted in SNPTEST-v2.5.4-beta[75]. Inference was limited to genotyped and imputed variants with SNPTEST Info scores of 0.4 or higher, with Hardy Weinberg equilibrium p-value > $5x10^{-8}$ for common variant analysis (minor allele frequency > 0.1). Inference rare variants, SNPs with MAF ≤ 1%, was further restricted to variants with an estimated minor allele count (SNPTEST Info score multiplied by minor allele count) of 10 or higher in each analysis sub-cohort.

The UK Biobank

Summary statistics from the analysis of the interim data from the UK Biobank (UKB) were utilized in our meta-analysis. These results have been previously reported by Warren et al[12]. Briefly, following central and study-specific quality control protocols, 140,886 empirically classified white individuals were analyzed for SBP, DBP, and PP traits. BP measures were averaged over two measures, and adjusted for medication use by adding 15 and 10 mmHg to

SBP and DBP, respectively. Linear models were adjusted for the top 10 principal components of ancestry, age, age^2, sex, an indicator for genotyping platform, and BMI.

Meta-Analysis of Discovery Datasets

Inverse-variance weighted fixed-effects meta-analysis of common variants across MVP subsets and summary statistics from UKB was performed using the METAL software. Genomic inflation factor was calculated, and λ_{GC} for the discovery from MVP were 1.195, 1.149, and 1.171 for SBP, DBP and PP, respectively, 1.303, 1.315, and 1.270 respectively, from UKB, and 1.275, 1.140, and 1.244, respectively, in the overall discovery analysis (Supplementary Fig. 3).

Selection of SNPs for Replication

Common Variants

For common variants, we considered for follow-up SNPs in loci non-overlapping with previously reported loci according to both an LD threshold of $r^2 \leq 0.1$ and a 1Mb interval. We obtained a list of these SNPs with p-value < $1x10^{-6}$ for any of the three BP traits, a minor allele frequency (MAF) ≥ 1%, and concordant directions of effect between UKB and MVP.

In silico replication summary statistics were provided for 942 SNPs by the International Consortium for Blood Pressure Genetics (ICBP) after meta-analysis of 77 individual participating cohorts for a total maximum of 299K individuals, who were genotyped and analyzed according to study-specific protocols. Additional replication results were provided from Vanderbilt University's BioVU EMR-linked biorepository, among which genotypes from the MEGA array and phenotype data were available from 17,277 participants. Discovery and replication data were combined using fixed-effects inverse-variance weighted meta-analysis implemented in METAL[76].

Rare Variants

We conducted an *in silico* replication analysis of 18 rare exonic SNPs from our discovery analysis in 417,143 participants from the BP-ICE consortium. SNPs were chosen for replication if they had a discovery p-value < $1x10^{-6}$, and a MAF < 1%.

Due to BP-ICE's emphasis on exonic rather than rare variation, we also pursued additional replication utilizing the larger release of the UKB data in order to capture non-exonic rare variation. Due to the inclusion of UKB data in the discovery set, for the second analysis we sought replication from variants suggestive only in MVP cohorts following meta-analysis as described above. 1,066 rare variants with p-value < $1x10^{-6}$ for any of the three phenotypes were selected for replication in 458,577 participants from UKB. Additional replication was provided as above from the BioVU MEGA genotyping, and all data was meta-analyzed using fixed-effects meta-analysis in METAL[76].

Classifying Results by Evidence for Association

For results that reach statistical significance of p-value ≤ $5x10^{-8}$ at any stage of the analysis, and that had consistent direction of effect between discovery and replication stages, we established three tiers of evidence that are annotated in results tables:

1) Genome-wide significance in the discovery stage, and Bonferroni-corrected significance in replication.

2) Genome-wide significance in the discovery stage, and p-value ≤ 0.05 in the replication stage.
3) All other associations reaching genome-wide significance across stages regardless of discovery or replication p-values.

Genetic Risk Score Construction

We constructed a genetic risk score (GRS) for each BP trait by calculating a linear combination of weights derived from the 140,886 participants from the UKB common variant analysis and index SNPs at each statistically significant locus observed in the MVP. Weighted GRS (w-GRS) were constructed for self-reported/administratively assigned white individuals in the MVP only.

Phenome-Wide Association Study Analysis

We performed a phenome-wide association study (PheWAS)[77,78] of GRS for each BP trait in MVP whites, leveraging the full catalog of ICD-9 diagnosis codes. We used logistic regression to separately model each of 1,813 PheWAS traits as a function of the three GRSs, adjusted for age, age^2, sex, BMI, and 10 PCs. We report the results from these analyses as odds ratios where the estimate is the average change in odds of the PheWAS trait per weighted BP-increasing allele. Multiple testing thresholds for significance were set to p-value ≤ 2.75×10^{-5}(0.05/1,813). All PheWAS analyses were conducted using the R PheWAS package[79].

S-PrediXcan Analysis

Genetically predicted gene expression was evaluated for the common variant subset with S-PrediXcan[80], a gene-level approach which estimates the genetically determined component of gene expression in a given tissue and tests it for association with SNP-level summary statistics. We utilized all three BP meta-analysis results for common variants and 44 tissues from GTEx[25] for this analysis, incorporating covariance matrices developed for European populations (1000 Genomes) as the majority of samples were European in origin.

For evaluating the accuracy of gene annotations for BP and HTN loci available in the GWAS catalog, significant S-PrediXcan results were considered if the expressed gene was within 1MB of the nearest gene to the top SNP (within 250kb, annotated using SNPDOC https://wakegen.phs.wakehealth.edu/public/snpdoc3/index.cfm). Previously identified loci annotations were considered "consistent" if the only significant expressed gene (in any tissue) matched the nearest gene, "partially consistent" if a significant expressed gene (in any tissue) matched the nearest gene and other expressed genes were also significant, and "inconsistent" if none of the significant expressed genes matched the nearest gene for the known locus.

Evaluation of Associated Loci as Kidney eQTLs

The GTEx resource lacks sufficient kidney tissue specimens to build predictive models for S-PrediXcan analysis, so we used the collection of kidney reference data that was recently described by Ko et al[28]. We considered all tiers of statistically significant SNPs in this analysis, and used COLOC[27] to calculate the posterior probability that the locus is both causal for the BP trait and causal for observed expression changes at a nearby gene. We present all SNP-gene pairs with a posterior probability of 0.95 or greater. For the genes implicated by the COLOC analysis, we also evaluated homologous genes in the single cell atlas of the mouse kidney, where expression levels are measured by single-cell RNAseq across 57,979 total mouse kidney cells

from 7 healthy mice[81]. This enables us to observe what cell types in the mammalian kidney express the genes where there is evidence for association between expression and BP traits. A single cell-gene matrix containing the UMI (unique molecular identifier) counts for 43,745 cells and 16,273 transcripts were generated from 7 normal mouse kidneys using 10x Chromium™ Single cell solution[81]. Original raw data files were deposited under GEO (GSE107585). The target genes of GWAS signals were identified by integrative statistical analysis of kidney eQTL result from 99 normal human kidney cortex samples[28] and GWAS signals using Bayesian colocalization approach *coloc*. Mouse orthologs for the human genes were found using Ensembl BioMart (http://www.ensembl.org/biomart/martview). Genes expressed less than 5% of the cell clusters were excluded from further analysis. To calculate the average expression level for each cluster, a z-score of UMI count was first obtained for every single cell. Then, we calculated the mean z-scores for individual cells in the same cluster, resulting in z-score for each gene and each cell cluster.

Evaluation of Drug Classes for Genes with Associations with Gene Expression

For genes with associations between GPGE and BP traits, we evaluated existing drugs for target gene and adverse drug events (ADEs), as well as cataloging whether the targets of known anti-hypertension drugs are detected, and identifying genes with potential for inhibitive drug targeting where increasing GPGE also increases BP and are currently targeted by a non-antihypertensive drug.

A list of medications with a primary indication for hypertension and a list of medications with adverse drug events (ADEs) for hypertension and hypotension indications were created using SIDER[82] and the DEB2 database[83]. Gene targets for antihypertension medications (Supplementary Table 14), medications targeting genes significant in S-PrediXcan analyses with positive effect sizes (Supplementary Table 15), and medications targeting genes mapped from significant GWAS signals (Supplementary Table 16) were queried using DGIdb[84] (http://www.dgidb.org/). Primary indications for medications targeting genes significant in S-PrediXcan analyses with positive effect sizes were compiled using the BIDD TTD database[85] (Supplementary Table 11; https://db.idrblab.org/ttd/). Genes targeted by antihypertension medications with a significant S-PrediXcan association in at least one tissue with at least one BP trait are presented in Supplementary Table 10 alongside the most significant S-PrediXcan result. S-PrediXcan genes associated with any trait, that have positive effect sizes in any tissue, and are targeted by a non-hypertension drug are presented in Supplementary Table 11 with the name of the drug and the primary indication for treatment. S-PrediXcan genes associated with any trait, targeted by a drug, and that have an ADE involving hypertension or hypotension are presented in Supplementary Table 12. Sample proportions presented in results and Supplementary Data were compared using a two-proportion z-test.

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